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Postnatal development of the light and electron microscopic features of basket cells in the hippocampal dentate gyrus of the rat

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Summary. Light and electron microscopic preparations were used to analyze the postnatal development of the basket cells of the rat dentate gyrus. The basket cells, located at the hilar border, were recognized in 2-day-old rats in Golgi preparations, where they displayed immature dendrites and a small axon arbor in the granule cell layer. At 5 days, the basket cells were found to have a large perikaryal cytoplasm, a round nucleus, an axon that forms symmetric synapses with granule cells, and dendrites and somata that are contacted by other axon terminals. The 10-day basket cells display more mature features, such as Nissl bodies and well-developed Golgi complexes. The basket cells from 16-day-old rats are mature in terms of their ultrastructural features, in that the nuclei are highly indented and display intranuclear rods or sheets, the perikaryal cytoplasm is packed with organelles, and the axon has developed an extensive arborization with the somata and dendrites of granule cells at the border with the molecular layer. This arborization will continue to expand as more granule cells are generated and added to the hilar border. These data correlate well with the immunocytochemical and biochemical development of GABAergic neurons in the dentate gyrus. Furthermore, the maturation of the structure of basket cells appears to precede the appearance of adult-like electrical activity in the hippocampus.

Key words: Basket cells – Hippocampus – Dentate gyrus – Rat – Postnatal development

Introduction

Recently, a large number of studies demonstrated different aspects of the postnatal maturation of the rat hippocampal formation, including its biochemistry, morphology and physiology (Bayer 1980; Coyle and Enna 1976; Harris and Teyler 1983; Lübbers and Frotscher 1988; Schlessinger et al. 1978; Skerritt and Johnston 1982; Swann et al. 1989; Wong and McGeer 1981). It was shown that recurrent inhibition appears around the 5th postnatal day in the CA 3 area of the Ammon's horn whereas similar inhibition appears several days later in the CA 1 area (Harris and Teyler 1983; Swann et al. 1989). Other data suggested that the biochemical maturation of GABA/benzodiazepine postsynaptic receptors was the cause for this delay (Janigro and Schwartzkroin 1988). Anatomical elements for a recurrent inhibitory circuitry exist in both the dentate gyrus and Ammon's horn of 5-day-old rats (Seress et al. 1989). However, Ben-Ari et al. (1989) and Mueller et al. (1984) demonstrated that the inhibitory neurotransmitter GABA elicits depolarization instead of hyperpolarization prior to the 5th day in rats, and between 6 and 10 days in rabbits. These data strengthen the importance of receptor maturation in regard to functional recurrent inhibition.

These developmental studies all confirm that a dramatic change in the biochemistry and physiology of the hippocampus occurs during the first two postnatal weeks. Consistent with this conclusion are data showing that GABA concentrations and GAD activity levels increase sharply during this time (Swann et al. 1989), as well as the number of GAD and GABA immunoreactive cell bodies (Seress and Ribak 1988). However, only a few studies have analyzed the postnatal development of the light and electron microscopic features of the local circuit neurons that are the source of inhibition in the hippocampal formation. In contrast, data for principal neurons have demonstrated the postnatal growth of dendritic trees, and an increasing number of spines and synapses of the granule cells in the dentate gyrus and the pyramidal cells of the CA 1 area (Crain et al. 1973; Minkwitz and Holz 1975; Pokorny and Yamamoto 1981; Seress and Pokorny 1981; Wenzel et al. 1981). This dichotomy between data for principal and local circuit neurons may be due to the fact that detailed descriptions of different local circuit neurons have appeared only

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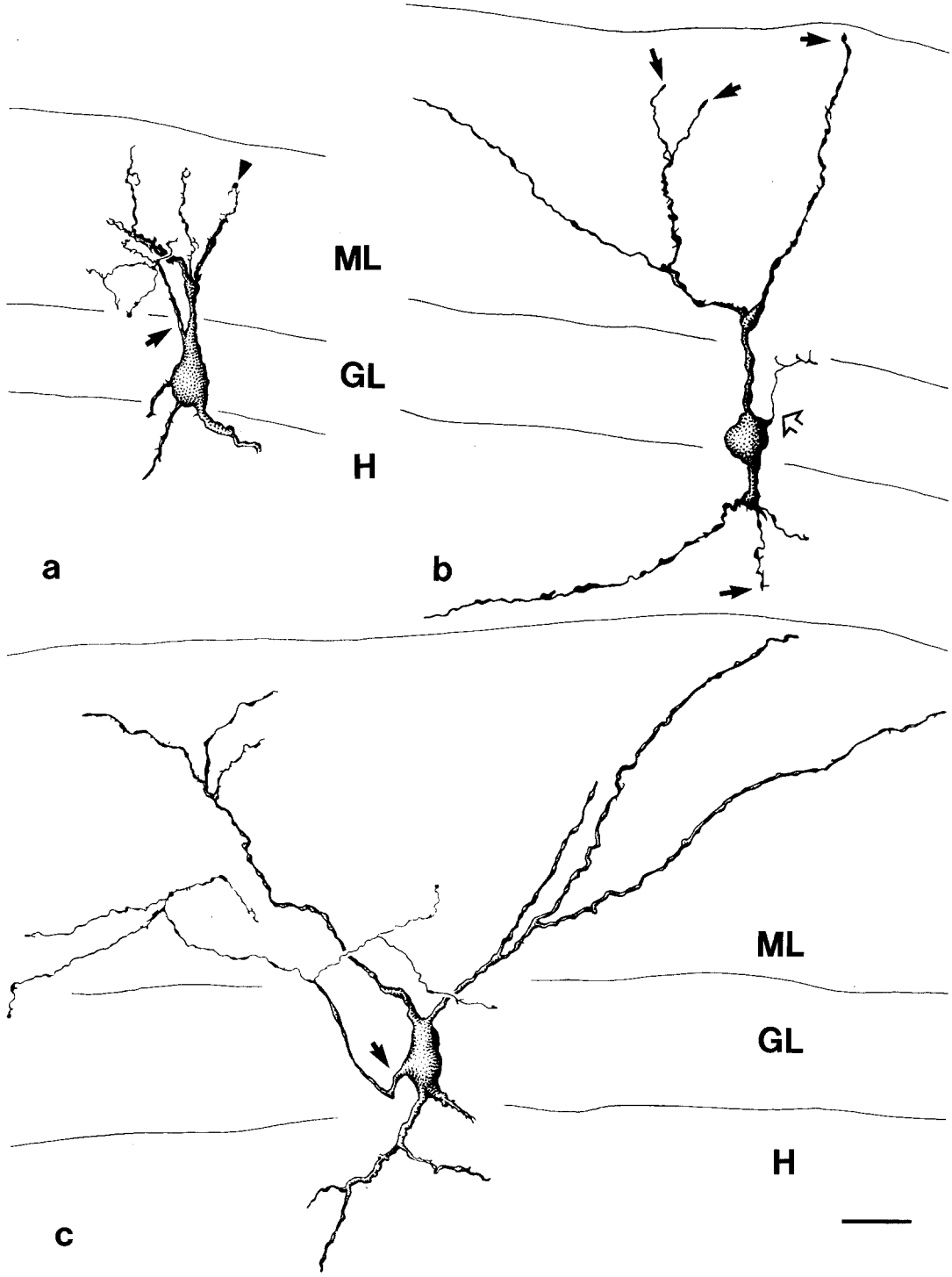
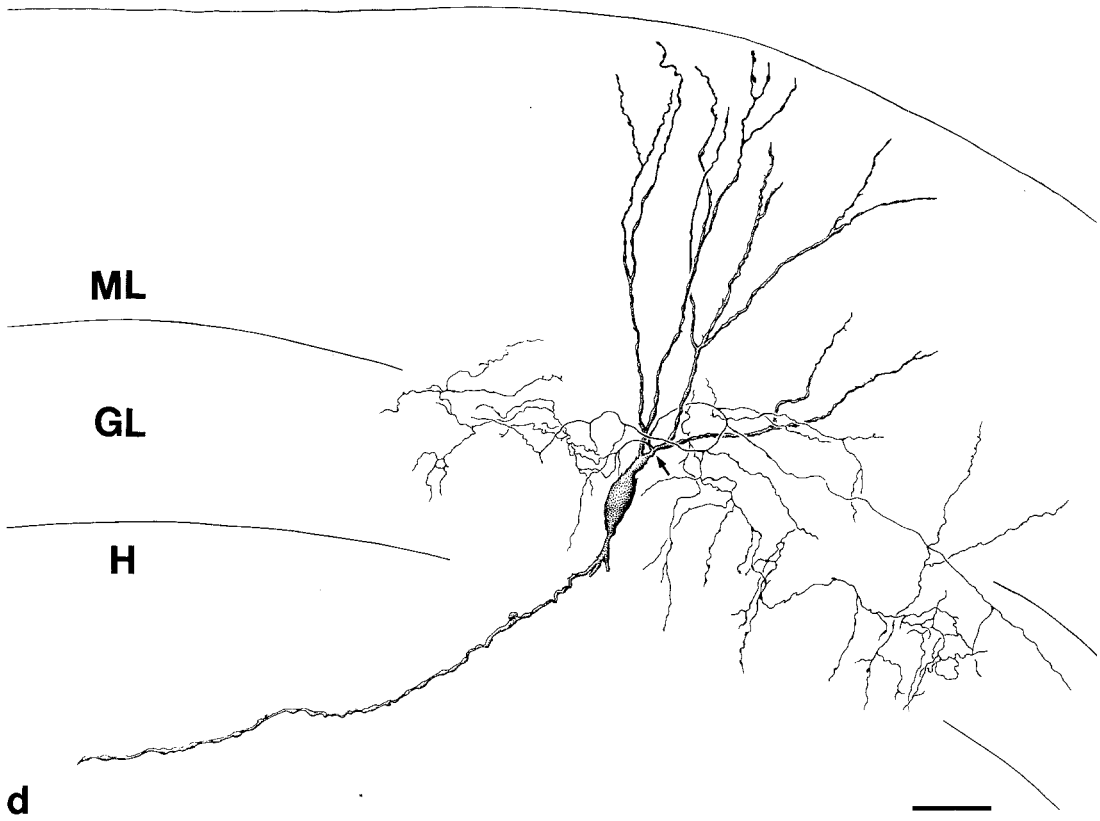


Fig. 1a–d. Camera lucida drawings of non-granule cells at the hilar (*H*) border of the granule cell layer (*GL*) of the dentate gyrus at various postnatal ages. **a** Shows a pyramidal type of basket cell from a 2-day-old rat. Basal dendrites protrude into the hilus (*H*) while the apical dendrites are in the molecular layer (*ML*). The dendrites are short and varicose, display thin filopodia and terminate in growth cones (*arrowhead*). The axon (*arrow*) arises from the apical dendrite and arborizes near the cell body. **b** Shows a fusiform type of basket cell from a 5-day-old rat. The dendrites are varicose and some of them terminate in growth cones (*arrows*). A small portion of the axon (*open arrow*) is impregnated. It originates from the cell body and gives off collaterals inside the granule cell layer. **c** Shows a pyramidal type of basket cell from a 10-day-old rat. The basal dendrites in the hilus (*H*) are short, but the apical dendrites in the molecular layer (*ML*) terminate at/or near the pial surface. These dendrites are smooth and varicose. The axon (*arrow*) originates from the cell body and gives off collaterals both inside and above the granule cell layer. Scale bar **a**, **b**, **c** 20 μm. **d** Shows a fusiform type of basket cell from a 16-day-old rat. The dendrites are thin, long, smooth and varicose. The axon (*arrow*) originates from an apical dendrite and richly arborizes among and above the granule cells. Scale bar 40 μm



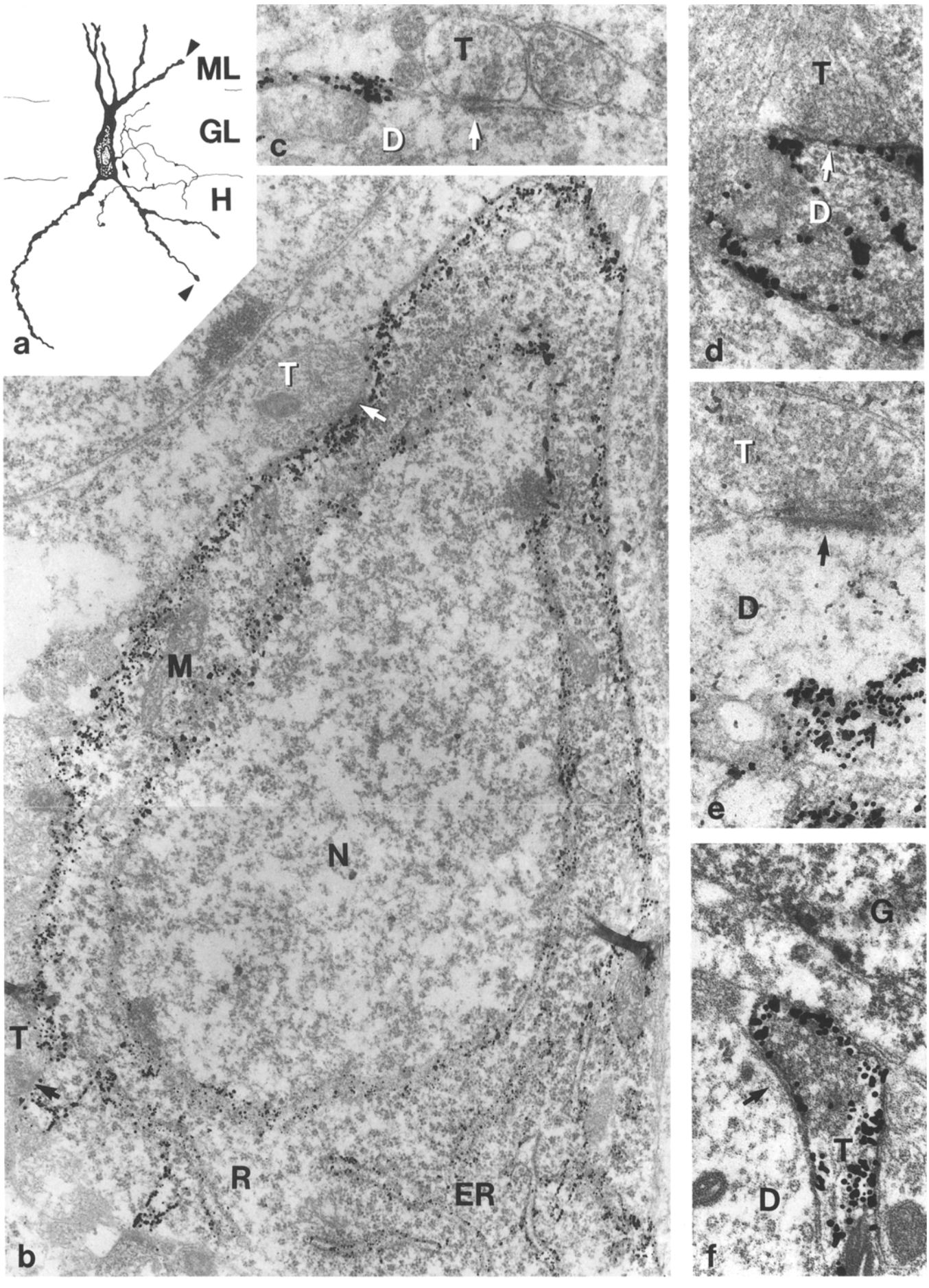
in recent years (Frotscher 1985; Frotscher and Zimmer 1983; Lübbers and Frotscher 1987; Ribak and Anderson 1980; Ribak and Seress 1983; Seress and Ribak 1985a). These combined Golgi-electron microscopic and immunocytochemical studies reinforced the previously known characteristics of local circuit neurons, such as smooth dendrites with numerous swellings, lack of spines (or very few spines) and axons that arborize extensively in the area around the cell body. In addition, these studies yielded information about the ultrastructure of these neurons which has been important for their identification in thin sections. Such characteristics include deep nuclear infoldings, intranuclear rods or sheets, and both symmetric and asymmetric axosomatic synapses for their cell bodies.

The aim of the present study is to describe the postnatal development of the basket cells of the rat dentate gyrus. In a companion paper, Lang and Frotscher (1990) describe the development of non-pyramidal cells in the CA 3 and CA 1 areas of the rat Ammon's horn. These studies provide morphological information about the maturation of non-principal cells in different parts of the hippocampal formation, and suggest that local circuit neurons develop at a similar rate throughout the hippocampal formation, in accordance with previous data (Seress and Ribak 1988).

Materials and methods

Sprague-Dawley rats of different ages (postnatal days 2, 5, 7, 9, 10, 12, 14, 16, 20 and adult) were fixed under ether anesthesia by intracardiac perfusion with a single solution containing 4.0% paraformaldehyde, 1.25% glutaraldehyde and 0.002% calcium chloride in a 0.12 M phosphate buffer at pH 7.2. Brains of two animals from each age group were processed for conventional electron microscopy. Blocks of the dentate gyrus were obtained by using a razor blade, then they were osmicated, dehydrated and embedded in Epon. Semi-thin 2–3-μm sections were cut in the horizontal plane for orientation, and then thin sections were taken of critical structures. All sections were stained with uranyl acetate and lead citrate before examination with an electron microscope. One brain in each age group was embedded in paraffin and sectioned for 10-μm-thick sections. These sections were stained with Cresyl violet according to the Nissl method.

Three animals from the age groups 2, 5, 10, 16 and 20 were fixed as described above, but then the brains were processed for the combined Golgi-electron microscopic method according to Fairén et al. (1977). The entire brain was rinsed, cut in the frontal plane in three blocks and placed into a solution containing 0.2% OsO_4 and 2.4% $K_2Cr_2O_7$ and kept in the dark at room temperature for 6 days. The tissue was then washed briefly in 0.75% $AgNO_3$ and stored in this solution for 3 days. Following impregnation, the brains were passed through 20, 40, 60, 80 and 100% solutions of glycerol, embedded in agar and sectioned. Sections were cut at 100 μm, collected on slides, coverslipped with 100% glycerol and examined with the light microscope. Golgi-impregnated basket cells at the hilar border of the granule cell layer were drawn with a Zeiss microscope equipped with a drawing tube. The sections containing these cells were hydrated through a series of glycerol solutions and placed into a chilled (4° C) 0.05% gold chloride solution for about 60 min with agitation. After three rinses in cold distilled water, they were placed into cold 0.05% oxalic acid for 2 min, brought to room temperature and placed into a 1% solution of sodium thiosulphate for 1–1.5 h. The sections were then rinsed in distilled water and examined with a light microscope to confirm the presence of the de-impregnated somata, dendrites and axons. The sections were then processed for electron microscopy using the routine schedule described above. Three or four basket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cells at the hilar border of the granule cell layer were examined from each age group.

The quantitative analysis of axosomatic synapses of granule cells used a goniometer stage to tilt the grids for the identification of synapses and their type. Synapses were classified as symmetric or asymmetric using the established criteria of postsynaptic density, width of the synaptic cleft and the shape of synaptic vesicles. One hundred cells were examined at both the hilar and molecular layer borders in all age groups. Statistical tests were used to determine significant differences between the examined ages.

Results

The basket cells of the dentate gyrus of rats have been described in a number of studies (Amaral 1978; Lorente de Nó 1934; Ramón y Cajal 1911; Ribak and Seress 1983; Seress and Ribak 1983). In the latter two studies, five types of basket cell were described in Golgi, immunocytochemical and Nissl preparations. Three of these cell types are found at the hilar border of the granule cell layer. They include the pyramidal, fusiform and horizontal cells. The most common type of basket cell is the pyramidal type that has one apical dendrite in the molecular layer, and two basal dendrites entering the hilus from the base of its pyramid-shaped soma. The fusiform basket cell is oriented vertically in the granule cell layer with basal dendrites extending into the hilus. The horizontal cell is similar to the pyramidal cell type, except that it lacks one of the two basal dendrites. The two other basket cell types include one in the molecular layer and another in the outer granule cell layer that is referred to as an inverted basket cell. The observations described below for the developing dentate gyrus are based on the two most common basket cell types that are found at the hilar border, the pyramidal and fusiform basket cells.

Light microscopy

Golgi preparations of 2-day-old animals showed that basket cells have larger cell bodies than granule cells. At this age, the dendrites are short and stubby with growth cones at their ends (Fig. 1a). Dendrites arise from the apical and basal poles of the cell body. Usually only a short portion of the axon is visible, but it is clearly different than the axons of granule cells because it originates from the apical part of the cell body, and gives off side branches in its immediate vicinity (Fig. 1a).

In 5-day-old animals, the basket cells appear similar to those found in adult animals, except that their dendrites are much shorter. The dendrites of 5-day-old basket cells are generally smooth and varicose. At the tips of these dendrites, growth cones are clearly visible (Fig. 1b). The axon is more developed than at the earlier age (Fig. 1b and Fig. 2a).

At 10 postnatal days the basket cells are more mature. They have much longer dendrites, that extend into both the molecular layer and the hilus, although some dendrites still display growth cones (Fig. 1c).

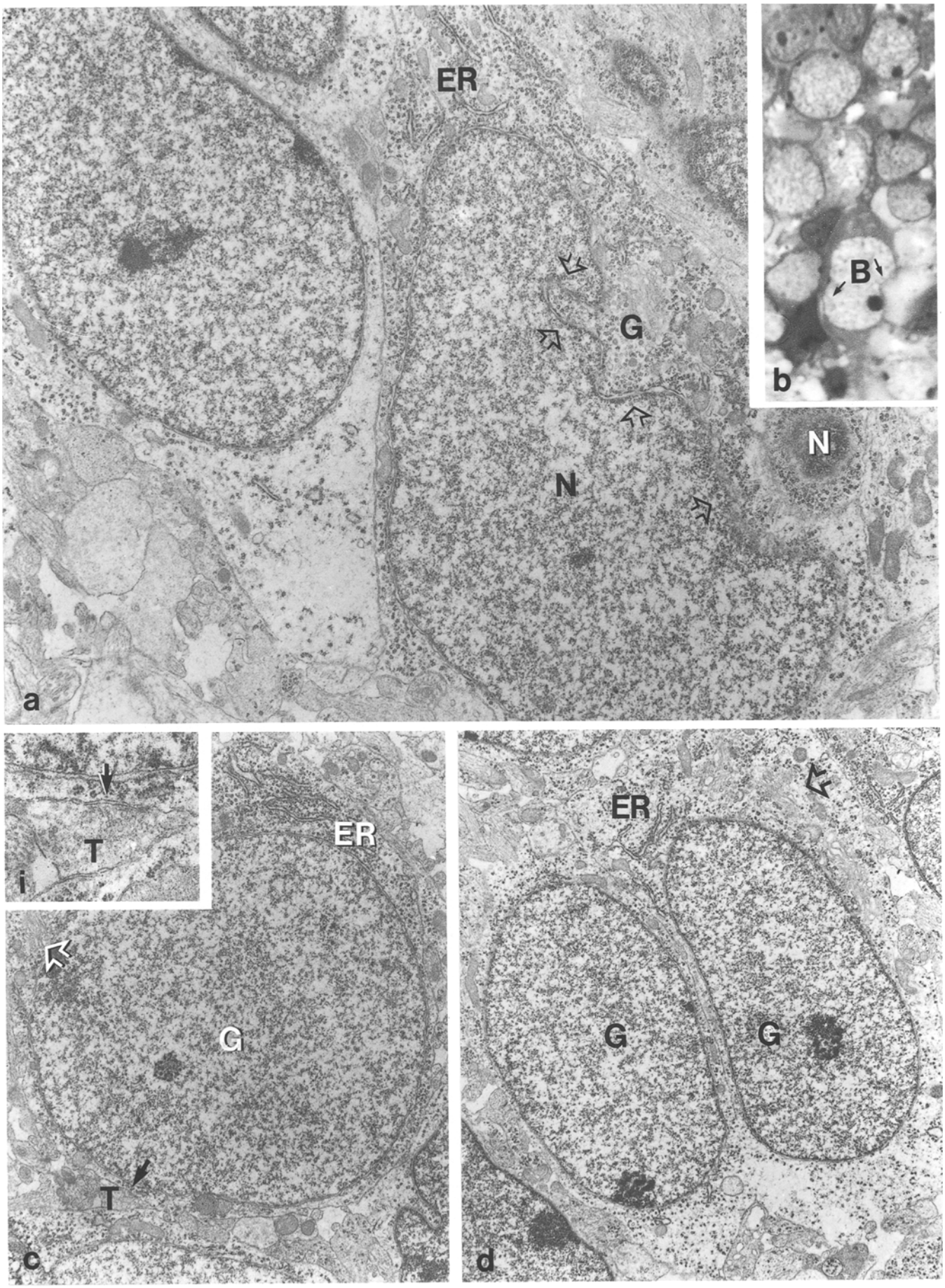
By postnatal day 16, the basket cells have reached the appearance of those found in adult animals. They have long, varicose smooth dendrites, and an extensively branching axon inside and above the granule cell layer (Fig. 1d). The cell body is larger than that found at earlier ages, and appears to be the same size as that found in adult animals.

The growth in the amount of perikaryal cytoplasm was also followed in Cresyl violet-stained preparations and semi-thin sections. In both preparations the increased staining of the cytoplasm was indicative of an increase in the amount of granular endoplasmic reticulum. In a few cases, accumulations of Nissl staining could be observed in 5- and 7-day-old basket cells (Fig. 3b), but basket cells with significant Nissl staining were regularly observed, and could thus be distinguished from granule cells after 10 postnatal days (Fig. 5b). By 12 and 14 postnatal days, basket cells appeared the same in individual Nissl-stained sections of the dentate gyrus as they did in adult animals. The maturation of the Nissl staining of basket cells is consistent with the development of Nissl bodies in electron microscopic preparations (see below).

Although most dendrites of developing basket cells are smooth and aspinous, spine-like appendages are occasionally found on these dendrites, but they are few in number. In one case, a basket-like cell in the upper granule cell layer from a 16-day-old animal was observed to display numerous spines on its apical dendrites in the molecular layer (Fig. 7a). The size of its cell body, the presence of a basal dendrite and an arborizing axon in the granule cell layer suggest that this neuron is a type of local circuit neuron. However, spiny variations of basket cells have not been described in adult animals so possibly these spines represent a transitional developmental stage.

In addition to spiny basket cells, granule cells displaying basal dendrites were observed during the first two postnatal weeks (Fig. 8a). These granule cells ap-

Fig. 2. a Camera lucida drawing of a pyramidal type basket cell in the granule cell layer (*GL*) of the dentate gyrus of a 5-day-old rat. It has apical dendrites in the molecular layer (*ML*) and basal dendrites in the hilus (*H*). The axon (*arrow*) arborizes near the cell body. Note the growth cones (*arrowheads*) on the tips of some of the dendrites. ×350 **b–f** Electron micrographs of the basket cell shown in **a**. **b** Shows the soma that contains a large and ovoid nucleus (*N*). The cytoplasm is sparse in organelles, containing free ribosomes (*R*), scattered cisternae of granular endoplasmic reticulum (*ER*) and a few mitochondria (*M*). Terminals (*T*) form synapses (*arrows*) with the apical and basal parts of the cell body. ×22500 **c** Shows a portion of a basal dendrite (*D*) of this basket cell where gold-labeling is found between the mitochondrion and the dendritic membrane. An apposing axon terminal (*T*) forms a synapse (*arrow*) with the dendrite at a site where the gold label is lacking. ×33000 **d** Shows a part of a gold-labeled apical dendrite (*D*) that displays a synapse (*arrow*) with an axon terminal (*T*). ×40000 **e** Shows another portion of a gold-labeled basal dendrite (*D*) that displays an asymmetric synapse (*arrow*) with a large axon terminal (*T*). ×40000 **f** Shows a gold-labeled axon terminal (*T*) that is apposed by a granule cell body (G) but forms a symmetric synapse (*arrow*) with an adjacent dendrite (*D*). ×40000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pear similar to others found in the rat, with the exception that they usually have one thin basal dendrite that extends into the hilus. Such cells are absent in adult rodents.

Electron microscopy

Previous studies have described the electron microscopic features of basket cells from adult rats (Lübbers and Frotscher 1987; Ribak and Anderson 1980; Ribak and Seress 1983; Seress and Ribak 1984). A brief review of these features is provided as a background for the identification of basket cells in the developing dentate gyrus. Although Golgi-electron microscopic preparations were used for the identification of basket cells in 5-, 10- and 16-day-old animals, somata of basket cells were easily identified in normal electron microscopic preparations by some of their distinguishing features at these and intervening ages.

Briefly, the five types of basket cell share the same ultrastructural features (Ribak and Seress 1983). The nuclei of all basket cells display deep nuclear infoldings and intranuclear rods or sheets. The size of these nuclei is much larger than the granule cell nuclei. The perikaryal cytoplasm of basket cells is also more substantial than that of granule cells, and it contains a large number of organelles. Cisternae of granular endoplasmic reticulum are numerous and they typically group to form Nissl bodies. The Golgi complex is well developed in many regions of the cytoplasm. Microtubules, mitochondria and free ribosomes are also numerous. The axon terminals that form synapses with basket cells make both asymmetric and symmetric synapses. These two types of synapse are also observed along the smooth surface of basket cell dendrites. Finally, the axon terminals that arise from basket cells contain pleomorphic synaptic vesicles, and form symmetric synapses with granule cell bodies, dendrites and occasionally with dendritic spines.

Postnatal days 2 to 8

Although light microscopic Golgi preparations showed basket cells at 2 days, electron microscopic preparations of cells from this age were not useful for analysis because the tissue was poorly preserved. The earliest stage of development when basket cells could be recognized in semi-thin and thin sections from normal tissue was 5 days (Fig. 3b).

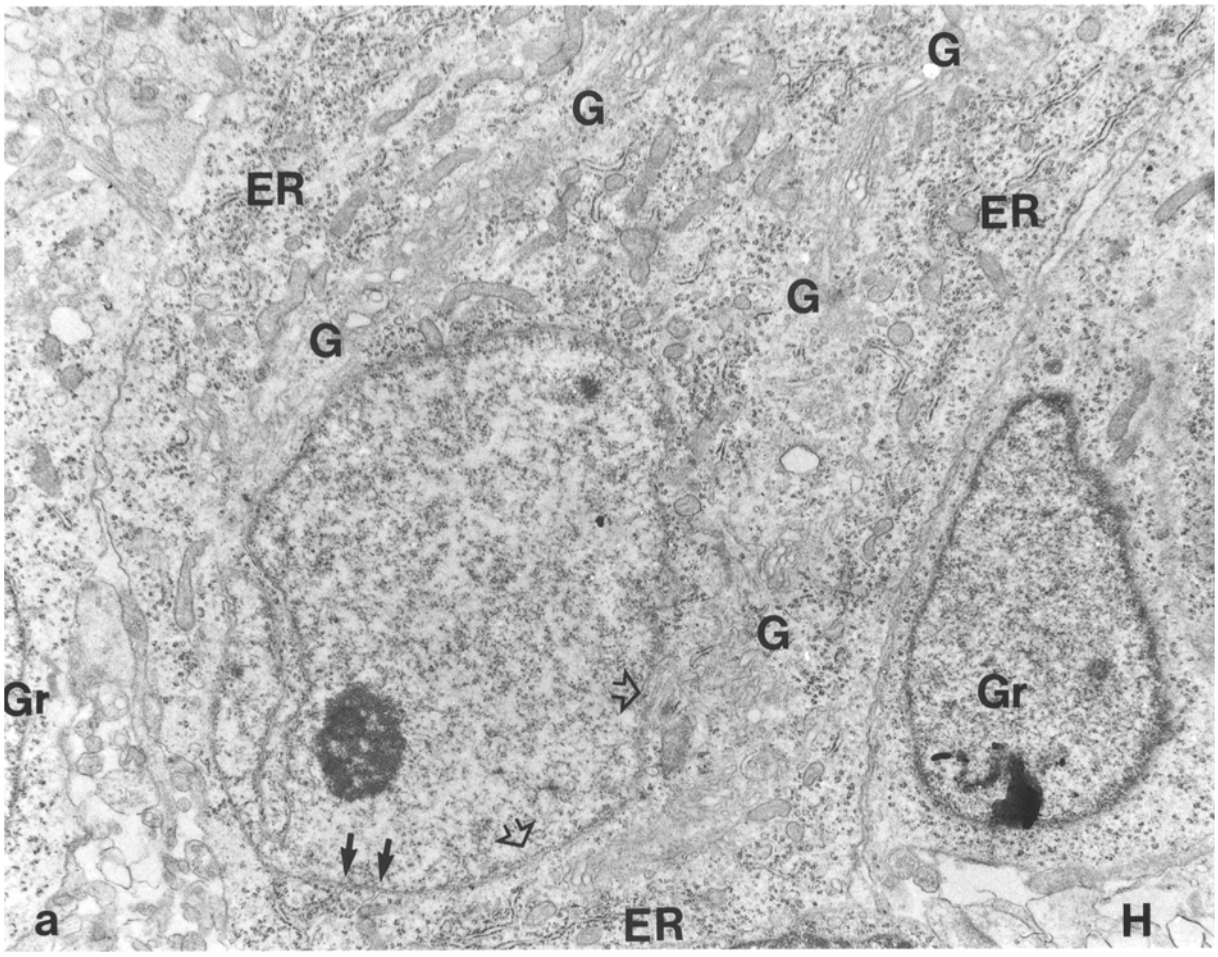
In electron microscopic preparations from 5-day-old animals, basket cells located at the hilar border of the granule cell layer show a large cell body when compared to granule cells. The perikaryal cytoplasm of basket cells is relatively small, and contains fewer organelles than are found in basket cells of adults (Figs. 2b, 3a). The organelles at this age include large numbers of free ribosomes that form clusters, and scattered cisternae of granular endoplasmic reticulum that never form the well-organized parallel stacks considered to be a Nissl body. The Golgi complex is also present and is usually located toward the apical part of the cell nucleus (Fig. 3a). However, at this age it is smaller and less developed than in adults. A few small mitochondria are scattered around the cytoplasm.

Terminals of unknown origin form asymmetric synapses with the basket cell body (Fig. 2b) and its dendrites located in both the molecular layer and the hilus (Figs. 2c–e). However, the number of such synapses is small. In serial sections of the cell body of identified basket cells there are no more than 2 to 3 synapses per cell body per section. Synapses appear to be more frequent along the surfaces of basket cell dendrites.

The basket cells have a large, ovoidal-shaped nucleus that shows infoldings of various degrees (Fig. 3a). Intranuclear rods are never found in the cell nucleus of any of the examined basket cells throughout this 2 to 8-day period.

The axon terminals of the basket cells form symmetric synapses with granule cell bodies and dendrites (Fig. 2f). The axon terminals are considered to be immature because they contain few mitochondria and a small number of vesicles. The granule cells that are targets of these axon terminals are located at the border with the molecular layer (Figs. 3c, i). In the 2- and 5-day-old animals, axosomatic symmetric synapses on granule cells are infrequent (0.23 and 0.7 per cell, respectively) because less than one quarter of adult numbers are observed for cells at the border with the molecular layer (Table 1). However, a significant increase in their numbers occurs at 7 days as compared to 5 days, ($P < 0.001$ unpaired t-test). In contrast, the granule cells located in the middle of the granule cell layer or at the hilar border hardly ever display axosomatic synapses

Fig. 3. a Electron micrograph of a large non-granule cell at the hilar border of the granule cell layer of the dentate gyrus from a 5-day-old rat. This cell is not impregnated and therefore the cytoplasmic structure is not obscured by gold particles. The cytoplasm contains free ribosomes, scattered cisternae of the granular endoplasmic reticulum (*ER*) and a small Golgi apparatus (*G*). Stacks of cisternae of granular endoplasmic reticulum or Nissl bodies are not visible at this age. The nucleus (*N*) is ovoid and displays deep nuclear infoldings (*open arrows*) but no intranuclear rod. The granule cell to its left is in a developmental stage where it displays a perikaryal cytoplasm practically devoid of organelles. Such granule cells are located characteristically at this site throughout the first three postnatal weeks. ×9000 **b** Photomicrograph of a semi-thin section that shows a non-granule cell (*B*) in the same position as that shown in Fig. 3a. Note the characteristic infoldings of the nucleus (*arrows*). ×1500 **c** Electron micrograph of a granule cell (*G*) found at the border with the molecular layer from a 5 day-old animal. The cytoplasm displays parallel lamellae of granular endoplasmic reticulum (*ER*) and a Golgi apparatus (*open arrow*). An axon terminal (*T*) forms a synapse (*arrow*) with this cell body (*see insert*). ×6500 The insert (*i*) shows at higher magnification this terminal (*T*) and the axosomatic synapse (*arrow*). ×20000 **d** Electron micrograph of two apposed granule cells (*G*) at the hilar border of the dentate gyrus from a 5-day-old rat. These neurons are in a neuroblast stage of development that is characterized by an organelle-poor perikaryal cytoplasm. Only a few cisternae of granular endoplasmic reticulum (*ER*) and a Golgi apparatus (*open arrow*) are found in the apical part of the cytoplasm. The basal part of the cytoplasm contains mainly free ribosomes. ×6500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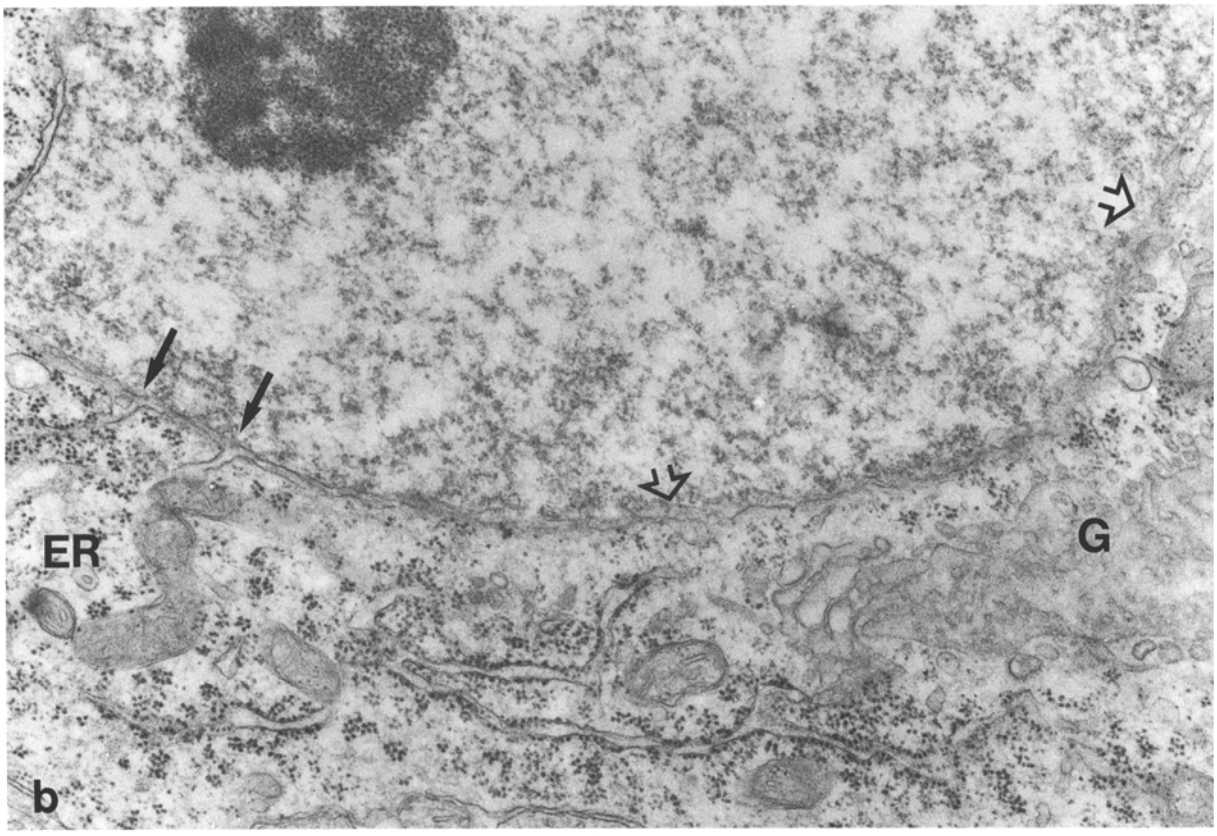
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(Fig. 3d). A survey of one hundred granule cells at the hilar border from 5- and 7-day-old rats showed that only 6 axosomatic synapses occur for 100 cells at each age. For 2-day-old rats no synapses were found for granule cells at this location (Table 1).

The granule cells at the border with the molecular layer are probably the ones that were generated earliest, as suggested by previous studies (Bayer 1980). They display a relatively well developed perikaryal cytoplasm that contains a large Golgi complex and a few cisternae of granular endoplasmic reticulum (Fig. 3c). In contrast to these granule cells, those in the middle part of the granule cell layer display a very thin cytoplasmic rim that is sparse in organelles. At the hilar border the granule cells are even more immature, and different developmental stages of these could be observed (Fig. 3d). Some of the latter are probably in a neuroblast stage, because they display a dark cell nucleus and a light, thin cytoplasm without organelles. Other granule cells in this location that are somewhat more mature, display more organelles in the perikaryal cytoplasm, but still show some features that are similar to neuroblasts, such as an organelle-poor cytoplasm at the basal part of the cell (Fig. 3d).

In 7-day-old animals the basket cells show similar features to those described for the 5-day-old animals. However, in some cases their perikaryal cytoplasm is considerably larger and contains more numerous organelles (Figs. 4a, b). The nuclear membrane of such basket cells shows frequent continuities with the intracytoplasmic membranes of cisternae associated with the endoplasmic reticulum and Golgi complex (Fig. 4b) suggesting a very active formation of these cytoplasmic organelles at this age. The nucleus is often infolded but lacks intranuclear rods.

Postnatal days 9 to 16

The cytoplasm of the basket cells during this period shows a significant development. In 9-day-old animals, the cytoplasm of basket cells contains a large number of cisternae of granular endoplasmic reticulum (Fig. 5a). Some of them form Nissl bodies at this age, and the number of such organized cisternae increases throughout days 12 to 16 (Figs. 5a, 6a). Also, the Golgi complex is larger and better organized. The size and number of mitochondria seem to increase throughout this period. The cell nucleus displays deep infoldings at all ages in this period, and intranuclear rods first appear at 12 days of age (Figs. 5e; 6a, b). These rods are always observed in basket cells from 16-day-old animals where they tend to be located near the nucleoli. The size and shape of the rods vary in the individual neurons (Figs. 6a, 7c).

The apical and basal dendrites are smooth, but varicose, and they are contacted by a large number of axon terminals that form both symmetric and asymmetric synapses. Compared to the 5-day-old basket cells, the frequency of axodendritic synapses is more than double in the 10-day-old animals. The distribution of the synapses is similar on the dendrites and cell body of the spiny non-granule cell of the 16-day-old rat. In this case, terminals do not form synapses with the spines even though they appose them. However, they do form synapses with the cell body and dendritic shafts (Figs. 7b, d).

The axons of basket cells in the 10- and 16-day-old animals form numerous symmetric synapses with both dendrites and cell bodies of granule cells (Fig. 5c, Figs. 6e, g). In the 16-day-old animal the gold-labeled axonal branches of the basket cell (Fig. 1d) could be traced in both the granule cell and molecular layers. Inside the granule cell layer the axon branches run along the surface of the cell bodies (Fig. 6d) and form symmetric synapses with them (Fig. 6e). In the molecular layer these branches often lie alongside the dendrites (Fig. 6f) where they form synapses (Fig. 6g) with them in a 20–30-μm-thick zone above the granule cells. Within the granule cell layer, most of the synapses are found in its upper half. The quantitative data are consistent with this finding, because the number of axosomatic symmetric synapses for granule cells at the molecular layer border reaches the adult level at 10 days and remains at this level at 16 days. In contrast, granule cells at the hilar border display infrequent axosomatic synapses (Table 1).

The granule cells in the outer half of the granule cell layer seem to be mature at this age. Their cytoplasm and nucleus show similar features as that found in adult granule cells. However, a large number of granule cells inside the granule cell layer and at the hilar border still display stubby, short dendrites that end in growth cones (Fig. 8a) although they also have spines. Basal dendrites are also observed on granule cells in this period (Fig. 8a). In the electron micrographs, granule cells with basal dendrites appear very immature, because they display a dark nucleus (Figs. 8b, c) and a thin, light cytoplasmic rim that contains free ribosomes and a few cisternae of endoplasmic reticulum (Fig. 8c). The basal dendrites show no spines, but form synapses with apposing axon terminals in the hilus (Figs. 8d, e). Also these

Fig. 4a, b. a Electron micrograph of a non-granule cell that is located among granule cells (*GR*) at the hilar border (*H*) of the dentate gyrus in a 7-day-old animal. Due to the plane of the section, a large portion of the cytoplasm is present. Lamellae of the rough endoplasmic reticulum (*ER*) are present in all parts of the cytoplasm, but they do not form the organized parallel bundles that could be called a Nissl body. The Golgi apparatus forms an extensive network (*G*) around the cell nucleus. The nucleus is round and contains a large nucleolus. The nuclear membrane shows a deep invagination near the nucleolus. A large portion of the nuclear membrane is in close contact with the granular endoplasmic reticulum (*solid arrow*) whereas another portion is apposed to a Golgi complex (*open arrow*). ×8250 **b** Enlargement of the portion of the nuclear membrane in **a** that shows a continuity (*solid arrows*) with cytoplasmic membranes of the granular endoplasmic reticulum (*ER*). *Open arrows* indicate regions of the nuclear membrane that appear to form small sacs or vesicles which are adjacent to the tubuli of a Golgi complex (*G*). This proximity suggests that these smooth membranes may join the Golgi complex. ×27000

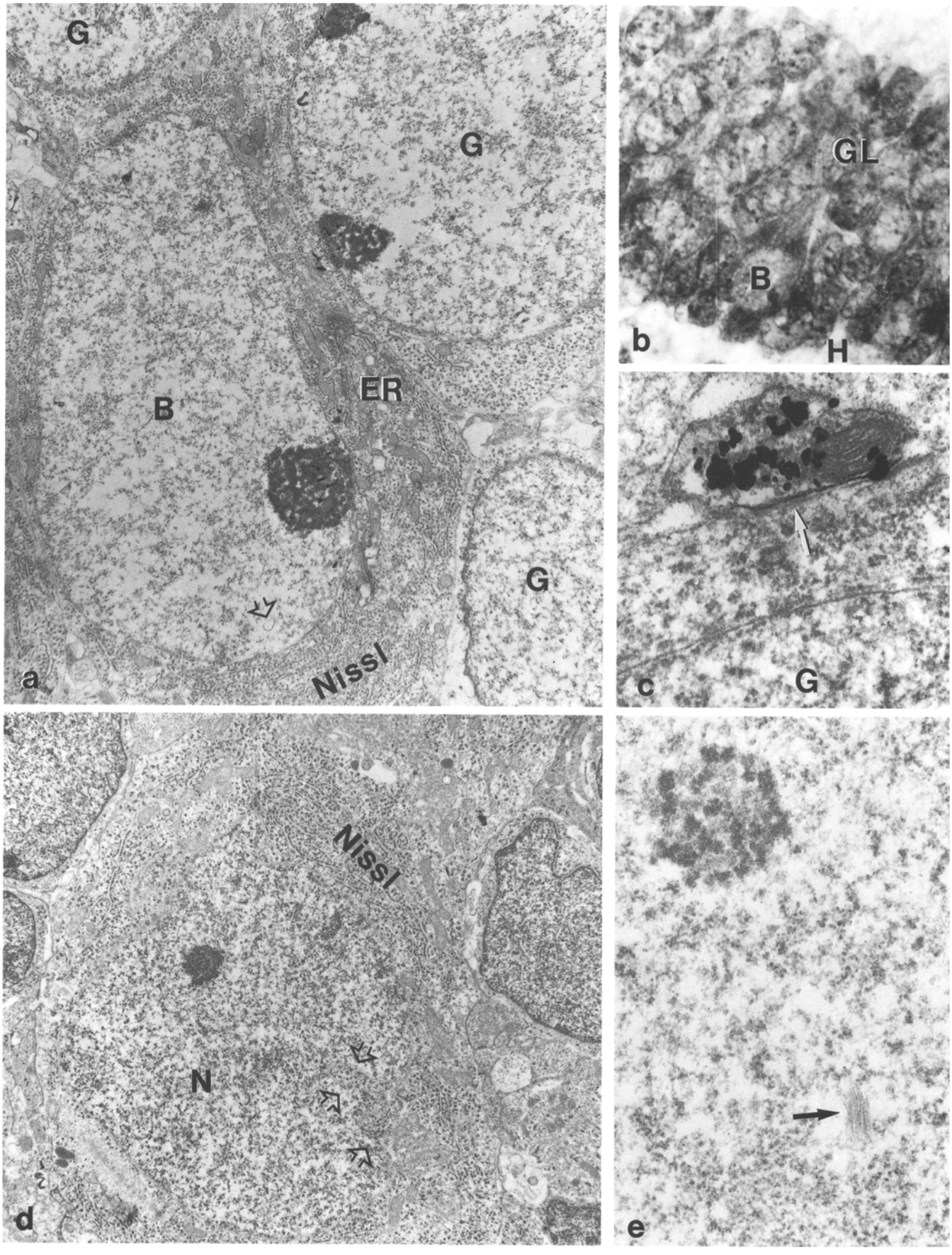
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Table 1. Development of axosomatic synapses of granule cells

Postnatal days	Total number of synapses for 100 cells		Number of symmetric synapses per cell ±SE		Percentage of asymmetric synapses	
	ML[a] border	Hilar border	ML[a] border	Hilar border	ML[a] border	Hilar border
2	24	0	0.23±0.05	0	4.2%	–
5	71	6	0.70±0.08	0.06±0.02	1.4%	–
7	127	6	1.25±0.16	0.06±0.02	1.6%	16.7%
10	289	9	2.67±0.21	0.09±0.03	7.6%	–
16	257	3	2.23±0.14	0.03±0.02	13.2%	33.3%
Adult	337	247	3.14±0.18	2.31±0.14	6.8%	6.5%

[a] ML, Molecular layer

dendrites show an ultrastructure similar to that of apical dendrites of granule cells.

Discussion

The major finding of the present study is that the ultrastructure of local circuit neurons of the dentate gyrus undergoes significant development throughout the first two postnatal weeks. This ultrastructural development of basket cells, a known GABAergic cell type, correlates well with the immunocytochemical and biochemical development of GABA- and GAD-containing neurons in the dentate gyrus (Seress and Ribak 1988; Swann et al. 1989). It also precisely precedes the appearance of adult-like electrical activity in the hippocampus (Leblanc and Bland 1979). Therefore, the ultrastructural development of the basket cells is closely related to an enhanced production of its neurotransmitters that is necessary for functioning in the adult rat.

Fig. 5. a Electron micrograph of a non-granule cell (*B*) at the hilar border of the granule cell layer in a 9-day-old rat. The nucleus is large, ovoid and has a large, single nucleolus. In serial sections, it also displays membrane invaginations but not intranuclear rods even though some intranuclear filaments (*open arrow*) are present. The perikaryal cytoplasm is large compared to that of adjacent granule cells (*G*). It contains many parallel cisternae of granular endoplasmic reticulum (*ER*) and in some places they form a Nissl body (*Nissl*). ×6600 **b** Photomicrograph of a non-granule cell (*B*) at the hilar border (*H*) of the granule cell layer (*GL*) in a 10-day-old animal. The cytoplasm of this neuron is stained darkly with Cresyl violet suggesting that at this age Nissl bodies are present in the perikaryal cytoplasm. Note that the granule cells show a very light or no cytoplasmic rim around their nuclei. ×1000 **c** Electron micrograph of a gold-labeled axon terminal of the impregnated 10-day-old basket cell shown in Fig. 1c. The terminal forms a symmetric synapse (*arrow*) with a granule cell body (*G*). The features of the nucleus and cytoplasm of this impregnated basket cell are similar to those of the non-impregnated basket cells at this age (Figs. 5a, b). ×20000 **d** Electron micrograph of a non-granule cell located at the hilar border of the dentate gyrus from a 12-day-old rat. The cell nucleus (*N*) is large and displays infoldings (*open arrows*). The perikaryal cytoplasm is also large and is rich in organelles. Nissl bodies (*Nissl*) regularly appear in their cytoplasm at this age. ×6600 **e** Serial section of the same cell nucleus shown in **d**. In this section an intranuclear rod appears (*arrow*) near the nucleolus. ×29000

Perikaryal cytoplasm

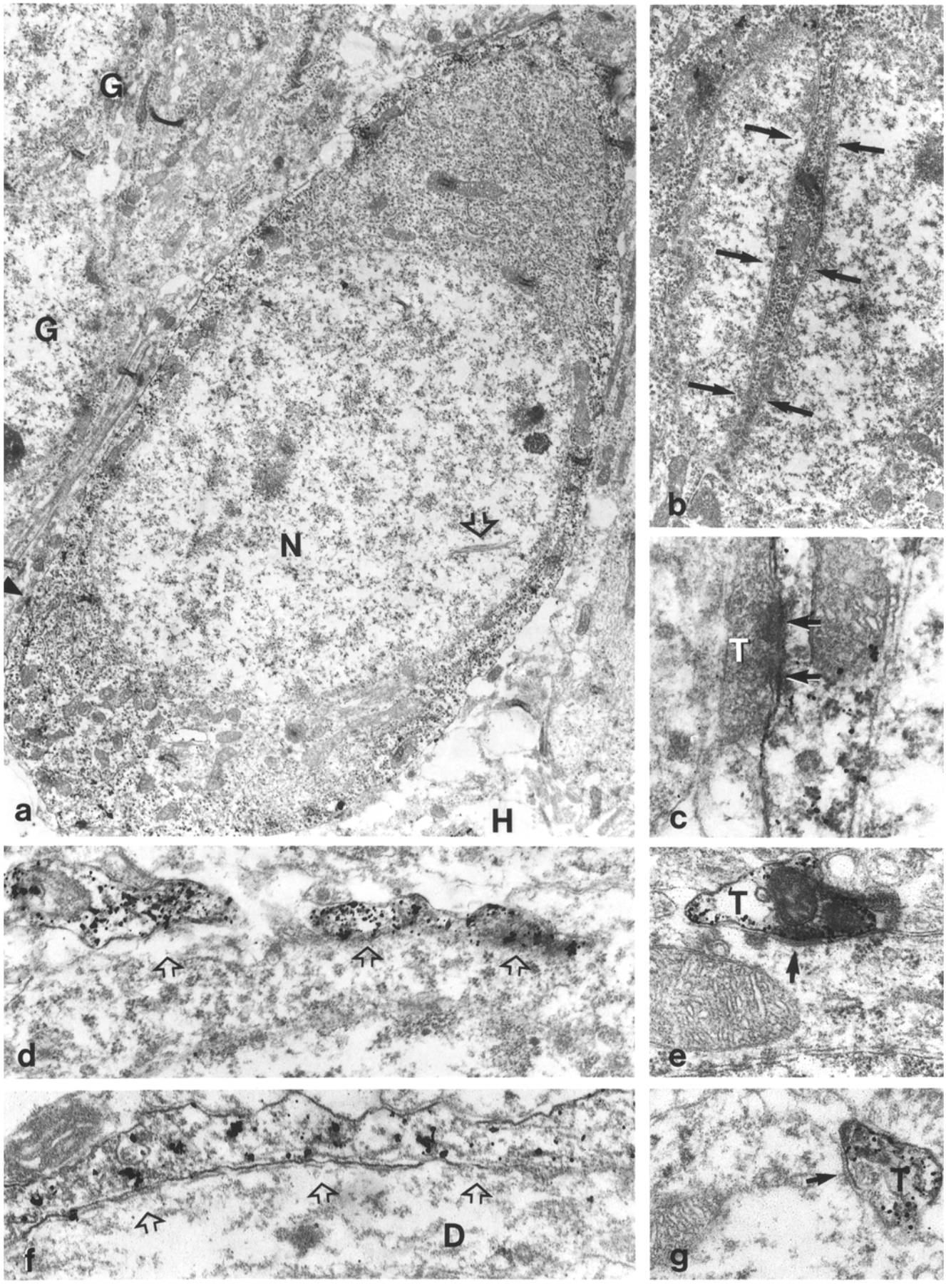
As stated above, the neurotransmitter found in all types of basket cell in the dentate gyrus is GABA (Ribak et al. 1978; Seress and Ribak 1983). However, some basket cells also colocalize neuropeptides, such as cholecystokinin, somatostatin or vasoactive intestinal polypeptide (Kosaka et al. 1985, 1988; Sloviter and Nilaver 1987).

It is generally accepted that the production of secretory or "export" proteins is the main function of the granular endoplasmic reticulum (Ghadially 1982). It is also known that cells which produce a protein-rich secretion are well endowed with granular endoplasmic reticulum. In the neurons, the basophilic bodies described in light microscopic preparations as Nissl bodies are revealed to be areas within the perikaryal cytoplasm that contain parallel cisternal arrays of granular endoplasmic reticulum and clusters of polyribosomes (Ghadially 1982; Peters et al. 1976). A perikaryal cytoplasm rich in granular endoplasmic reticulum and Nissl bodies is a characteristic of basket cells of the adult dentate gyrus (Ribak and Seress 1983). Therefore, the first occurrence of Nissl bodies in the perikaryal cytoplasm of 9-day-old rats suggests a growing capacity for protein synthesis from this age.

It is interesting to note that the cytoplasm of immature basket cells and newly born granule cells displays large numbers of free ribosomes and clusters of polyribosomes. It is well known that most of the protein required for endogenous cellular needs is produced by polyribosomes. Populations of fast-growing cells, such as embryonic and tumor cells, are often characterized by numerous polyribosomes lying free in the cytoplasmic matrix (Ghadially 1982). Therefore, the dominance of free polyribosomes in young neurons of the dentate gyrus may serve the need of protein synthesis for components of the growing neurons. The Golgi complex also develops in parallel with the granular endoplasmic reticulum. However, a well-developed Golgi complex appears a few days earlier than the Nissl bodies.

Cell nucleus

In association with the maturation of the perikaryal cytoplasm of basket cells, there are ultrastructural changes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in their cell nuclei. During the first postnatal week, the nuclei of basket cells show infoldings of various degrees, and frequent continuations of their nuclear membrane with membranes of the cisternae of the endoplasmic reticulum. Since the perikaryal cytoplasm displays little or no cisternae of granular endoplasmic reticulum at this time, it appears that the nuclear membrane is a major source of cytoplasmic membranes. Continuities between the nuclear envelope and granular endoplasmic reticulum are rarely observed in neurons from adult brains, except during periods of activity-induced increases in protein synthesis (Pico and Gall 1989). This view conflicts with the notion of Porter (1961) who considered the nuclear envelope "a large lamellar unit of the endoplasmic reticulum enclosing the nucleus" and suggested that the nuclear envelope was formed from the endoplasmic reticulum.

Intranuclear rods and sheets are a characteristic feature of the nuclei of basket cells in the adult dentate gyrus (Ribak and Seress 1983). They have not been reported in granule cells (Seress and Ribak 1985b). During the development of basket cells, intranuclear rods first appear at 12 days, nearly 2 to 3 days after Nissl bodies are formed in the perikaryal cytoplasm. Thus, the intranuclear inclusions are probably the last of the adult features to develop in basket cells.

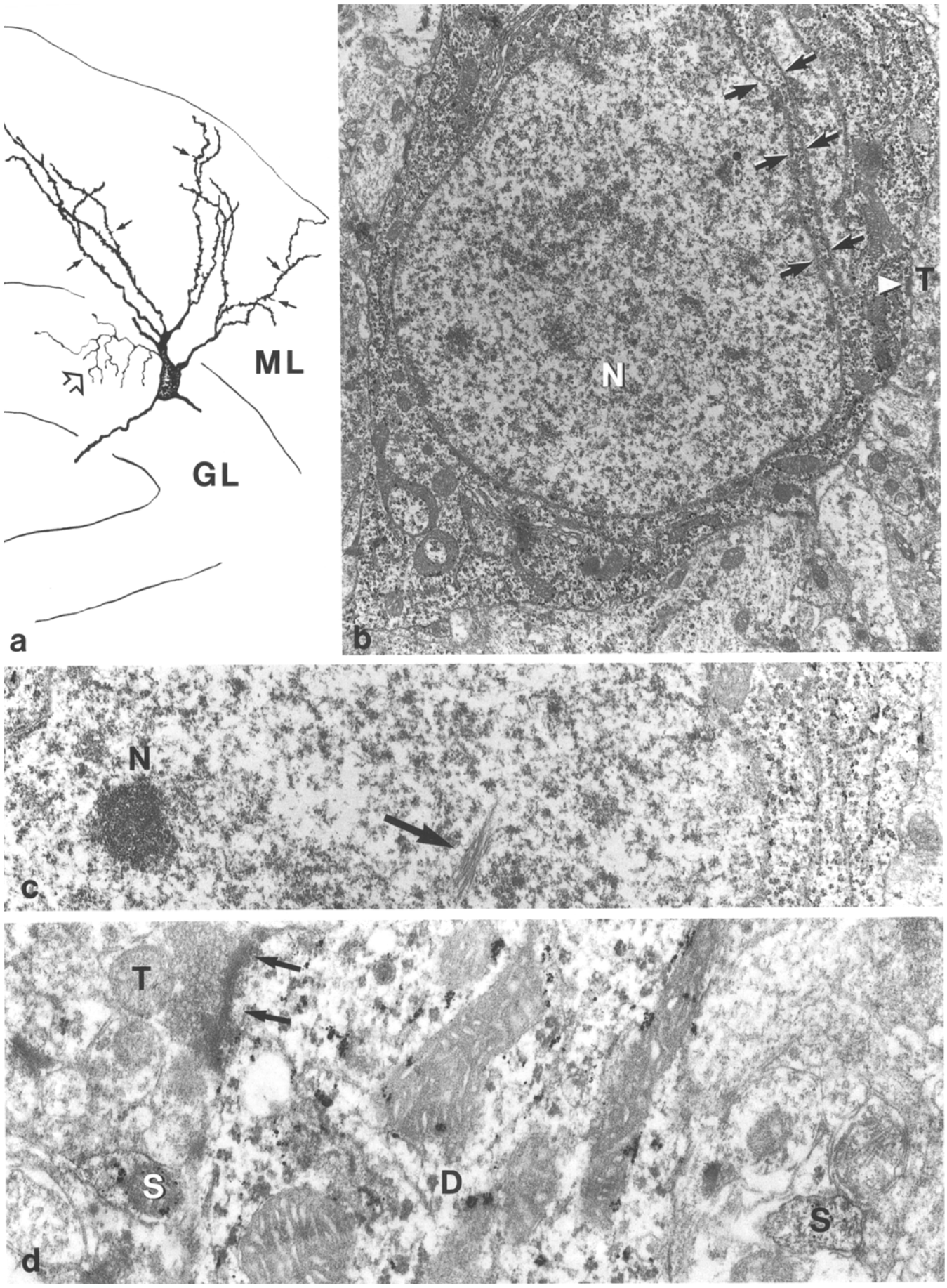
Since infoldings of the nuclear envelope and intranuclear bundles of fibrils have been described for neurons in the electron microscope by Siegesmund et al. (1964), there have been speculations about their functional role. Intranuclear rods have been found in neurons in different regions of the nervous system without any obvious connection to a distinct cell type (Curcio et al. 1984; Siegesmund et al. 1964). They have also been found in the cells of non-neural tissues and in poorly-differentiated neoplasm cells (Fukuda et al. 1987; Ghadially 1982; Payne and Nagle 1983). In fact, Payne and Nagle (1983) suggested that a quantitative evaluation of intranuclear rodlets, in combination with other ultrastructural features, may be a useful marker in the diagnosis of neoplastic tissue. Other studies have concluded that intranuclear rods are a sign of increased cellular activity, and may reflect the overall electrical activity of neurons (Curcio et al. 1984; Feldman and Peters 1972; Seïte et al. 1977). There are also suggestions that the rods are related to the aging of neurons (Miquel et al. 1983). The composition of intranuclear inclusions is not known, but crystallines, filaments and microtubuli are found to be some of their components (Seïte et al. 1973, 1975, 1977). Immunocytochemical observations suggest that the matrix of some of the intranuclear rods in tumor cells is similar to actin (Fukuda et al. 1987).

One thing in common with the results of the studies cited above is that they all relate the presence of intranuclear rods and sheets with active cells, including fast-firing neurons. In this regard, it should be noted that the basket cells of the dentate gyrus are known to be electrically and metabolically active neurons, and they belong to the fast spiking non-pyramidal cells of the hippocampus (Kawaguchi and Hama 1987; Kosaka et al. 1987). The appearance of intranuclear rods during the development of basket cells, and throughout adult life, probably reflects an increased activity of these neurons that is associated with a high turnover of neurotransmitter. The production of neuromodulatory peptides or neurotransmitter-synthesizing enzymes may cause an increased RNA transport from the nucleolus to the cytoplasm. The intranuclear rods and sheets may be related to actin filaments, and could be involved with the transport of RNA through the nucleus. The fact that rods and sheets in basket cells are associated with nucleoli, and tend to vary in size, suggests that they are nucleolus-related and activity-dependent.

Development of somal position

Basket cells at the hilar border of the dentate gyrus were chosen in this study to demonstrate the maturation of ultrastructural features of local circuit neurons. This group of basket cells represents about 70% of all the non-granule cells associated with the granule cell layer (Seress and Pokorny 1981). The most frequent types are the pyramidal and fusiform basket cells (Ribak and Seress 1983). In light and electron microscopic preparations, they are easily recognized by their characteristic shapes and their location among the granule cells bordering the hilus (Ribak and Seress 1983; Seress and Ribak 1983). The basket cells are generated together with other hilar neurons before the first granule cells are formed (Amaral and Kurz 1985; Lübbers et al. 1985). In the present study, the basket cells were found at the edge of the hilar region among the early generated granule cells in 2- and 5-day-old animals. Since they form and develop earlier than the majority of granule cells, they may play a role in the formation of the future granule cell layer. It remains to be determined whether the synapses formed by the axonal plexus of basket cells with the newly arrived granule cells are responsible for stopping further migration of granule cells toward the pial

Fig. 6a–g. Electron micrographs of the 16-day-old fusiform basket cell shown in the camera lucida drawing in Fig. 1d. **a** Shows its cell body at the hilar border (*H*) adjacent to two granule cells (*G*). At this age the basket cell displays similar features to those found in adults. The nucleus (*N*) is large and contains an intranuclear rod (*open arrow*) and infoldings (*see* **b**). An axon terminal (*arrowhead*) forms an axosomatic synapse. The perikaryal cytoplasm contains well-developed organelles, including Nissl bodies. ×7400 **b** A serial section shows a deep infolding (*arrows*) that appears to split the nucleus. ×9000 **c** Shows an axon terminal (*T*) in a serial section that forms an asymmetric synapse (*arrows*) with the cell body (*solid arrow* in Fig. 6a). ×37000 **d–g** Electron micrographs of the axon branches of the basket cell that form synapses with cell bodies and dendrites of granule cells. **d** An axon branch (*open arrows*) inside the granule cell layer that runs along the surface of a cell body. ×28000 **e** An axon terminal (*T*) that forms a symmetric synapse (*arrow*) with a granule cell body. ×34000 **f** An axon branch (*open arrows*) in the molecular layer that runs along the surface of a dendrite (*D*). ×30000 **g** An axon terminal that forms a symmetric synapse (*arrow*) with a dendrite. ×34000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surface. In 5-day-old animals, basket cells establish several synapses with the cell bodies and dendrites of granule cells at the border with the molecular layer, even though both of these cell types show immature features at this age (Seress et al. 1989; Wenzel et al. 1981). It has not yet been determined whether the basket cells at the hilar border form one cell type, with slight variations, or distinctly different cell types. It is possible that the pyramidal, fusiform and horizontal-shaped basket cells are variations of the same cell type, and the different shapes of these cells are simply caused by small differences in the packing of granule cells around them. However, it is also possible that they represent different cell types that contain different modulatory peptides or calcium binding proteins (Kosaka et al. 1987; Sloviter and Nilaver 1987). The two types that are found either at the border with the molecular layer (inverted type), or in the molecular layer, may reside at these sites because the migrating granule cells may have displaced them from their original site at the hilar border. The fact that all five types of basket cell display parvalbumin-immunoreactivity (Ribak et al. 1989) indicates that they share metabolic similarities as well as the structural similarity of having an axonal plexus in the granule cell layer and immediately above it (Ribak and Seress 1983; Seress and Ribak 1983).

Dendrites

The dendrites of basket cells in young and adult rodents are varicose and smooth with very few or no spines (Ribak and Seress 1983; Seress and Pokorny 1981). However, spiny basket-shaped cells have also been observed in Golgi stained preparations of young animals (Seress and Pokorny 1981). The present study confirms the existence of such neurons and extends the observation to the electron microscopic level. The ultrastructural characteristics of the spiny basket cell were similar to that of typical basket cells, except for the presence of spines on their dendrites. It is important to note that these spines did not form synapses with axon terminals, whereas axodendritic synapses were found on the dendritic shafts. Spiny local circuit neurons have also been described in the cortex of primate fetuses, suggesting that dendritic spines may be a common transitional feature of developing cortical basket cells (Lund et al. 1977; Marin-Padilla 1969).

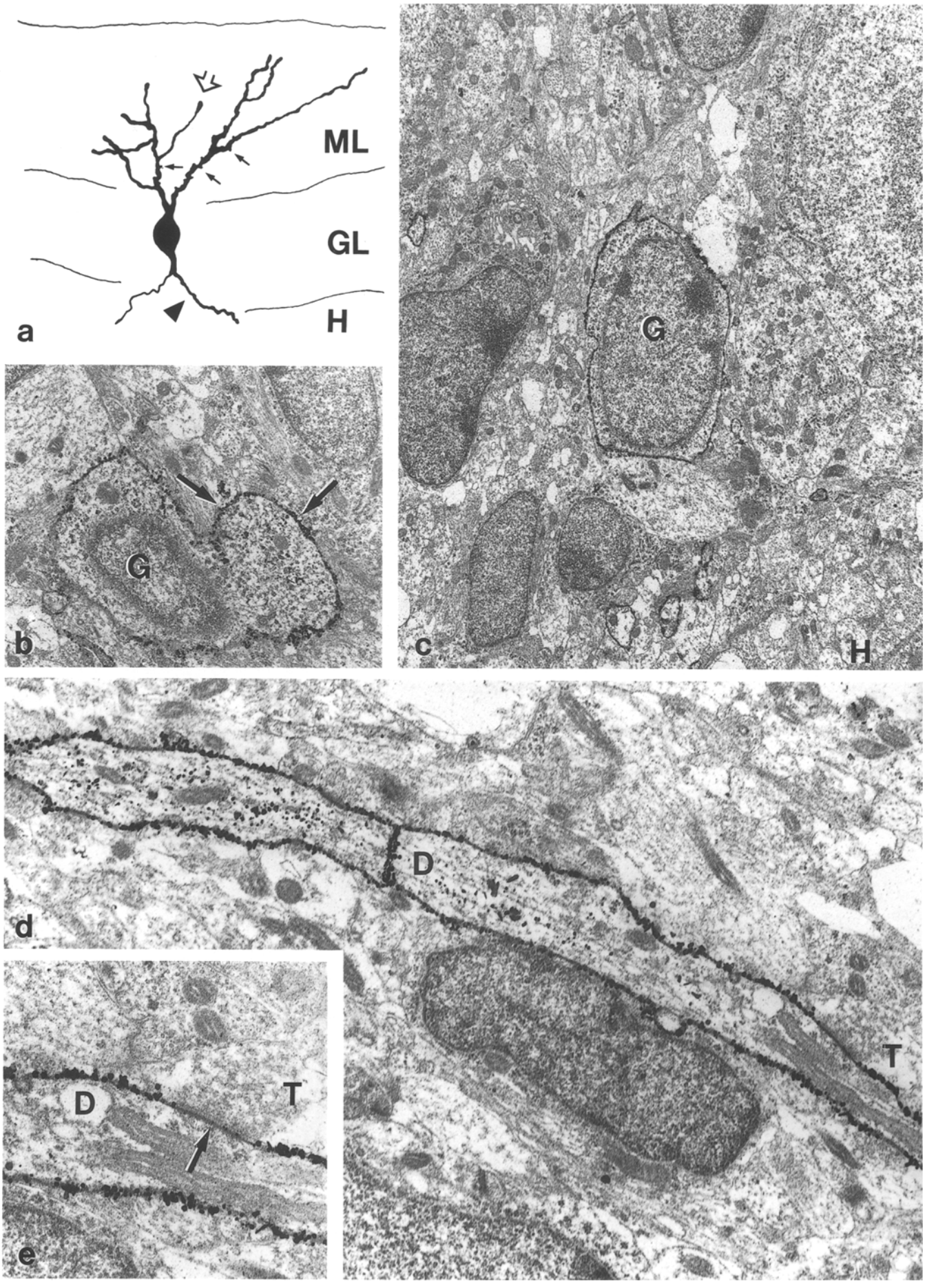
A similar, transitional feature is the appearance of basal dendrites on the developing granule cells of rats (Lübbers and Frotscher 1987; Seress and Pokorny 1981). The basal dendrites of some young granule cells deeply protrude into the hilus and they often have side-branches and spines. Axon terminals formed synapses with their dendritic shafts, but not with spines. In adult rodents, granule cells do not display basal dendrites. However, a large population of granule cells in primates displays basal dendrites (Seress and Mrzljak 1987). The basal dendrites of primate granule cells show spines that are similar to those on the apical dendrites, and axon terminals form synapses with these spines (Seress and Frotscher 1990). These results indicate that the transitional type of dendritic spine fails to form synapses with apposing axon terminals. However, the lack of synapses on these transitional processes may not be the cause of their disappearance during development, because transitional basal dendrites of granule cells establish normal axodendritic synapses that are probably functional. The growing number of mossy terminals in the hilus is also unlikely to be the cause of the dendritic regression, because there is evidence for persisting synapses between mossy fibers and basal dendrites of granule cells in primates (Seress and Frotscher 1990).

The transitional phenomena of developing neurons of the dentate gyrus suggest that the main characteristics of the individual neuron types are genetically determined, but the environment may also have a role in the formation of the final structure of a neuron. It is not known what these environmental factors are, but they could include neurotransmitters or modulators of certain afferents, the activity of the established synapses, and the number of synapses formed on the developing cell processes.

Axons

The axon of basket cells is apparent in the earliest Golgi stained preparations examined in this study, the 2-day-old rat. The axon arises from the apical dendrite and forms an arbor over the most superficial layer of granule cells in its vicinity. This group of cells at the molecular layer border represents the earliest born granule cells. Cells formed later are added to the granule cell layer at deeper levels. Thus, the first granule cells to be contacted by the basket cells are the first ones generated, and as later cells are generated they also become contacted by basket cell axons. Since the granule cells are generated over the first three weeks of postnatal development, the axonal arborization of basket cells continues its growth during this period. These ultrastructural results are supported by the immunocytochemical findings of Lübbers and Frotscher (1988) who showed that the GABAergic axonal plexus of basket cells in 5-day-old rats is limited to the inner molecular layer and the granule cells that border on the molecular layer. Granule cells at the hilar border at this age were not apposed

Fig. 7. a Camera lucida drawing of a spiny non-granule cell in the dentate gyrus of a 16-day-old rat. The neuron is larger than the granule cells, and has a basal dendrite that passes through the granule cell layer (*GL*) and enters the hilus. The apical dendrites are widely distributed in the molecular layer (*ML*). These dendrites display spines (*arrows*). The axon (*open arrow*) shows extensive branching near the cell body. ×400 **b–d** Electron micrographs of the neuron shown in **a**. **b** Shows the large cell body located among granule cells. The nucleus (*N*) displays nuclear infoldings (*arrows*) and an intranuclear rod (*see* **c**). Terminals (*T*) form asymmetric synapses (*arrowhead*) with the cell body. ×9000 **c** Shows a serial section that displays an intranuclear rod (*arrow*) near the nucleolus (*N*). ×17500 **d** Shows a portion of the apical dendrite (*D*) of the spiny basket cell. An axon terminal (*T*) forms an asymmetric synapse (*arrows*) with the dendritic shaft, but two gold-labeled spines (*S*) appear to lack synapses. ×32500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by GABAergic axon terminals, even though the adult displays many of these terminals around granule cells at the hilar border (Seress and Ribak 1983). A similar pattern of parvalbumin-containing terminals is also observed in the adult rat (Kosaka et al. 1987).

The quantitative data for axosomatic synapses of granule cells in the developing dentate gyrus indicate that basket cells first establish connections with the earliest generated granule cells, and then add further synapses to these cells as new synapses are formed with the deeper granule cells that are generated at later ages (Table 1). The number of symmetric synapses for granule cells at the molecular layer border in the 5- and 7-day-old rats is less than half of the number found for adults (Seress and Ribak 1985b). In contrast, the granule cells at the molecular layer border from 10-day-old rats show a significant increase in the number of axosomatic symmetric synapses as compared to 7-day-old rats ($P<0.001$, unpaired t-test). This increase raises the number to adult levels.

The data obtained from adult dentate gyrus in this study differ from data previously published. The total number of synapses was increased, whereas the number of asymmetric synapses was about half of the value indicated earlier (Seress and Ribak 1985b). The use of the $\pm 50°$ goniometer tilt has contributed to a more accurate identification of synapse type and number, because obliquely-oriented membranes are resolved, and cytoplasmic densities near the cell membrane can be distinguished from postsynaptic densities of the asymmetric synapses. The wide range in the frequency of asymmetric synapses between 10 days and adult ages was probably due to the limited sampling procedure of only 100 cells at each site.

It is interesting that the number of asymmetric synapses at the earlier ages is small relative to the adult data. This may reflect the late arrival of excitatory afferents from the commissural/associational system, the septum, or the recurrent axon collaterals of granule cells. In any event, it appears from the data presented in this study that the first group of axons to form synapses with the somata of granule cells arises from the basket cells. The data on the number of symmetric synapses in the molecular layer also show that they are significantly greater than the number of asymmetric synapses during early development (Crain et al. 1973). In a previous Golgi-electron microscopic study of adult rats (Ribak and Seress 1983), it was shown that identified basket cells form synapses with cell bodies and dendrites of granule cells. Therefore, it appears that the basket cells of the dentate gyrus form synapses with dendrites and cell bodies of granule cells before the arrival of a substantial number of excitatory afferent axons to the same cell population.

Fig. 8. **a** Camera lucida drawing of a granule cell with a basal dendrite in a 10-day-old rat. The cell body resides within the granule cell layer (*GL*). The apical dendrites branch in the molecular layer (*ML*) whereas the single basal dendrite (*arrowhead*) protrudes into the hilus (*H*). The apical dendrites are stubby, varicose and terminate in growth cones (*open arrows*). A few spine-like appendages are visible (*arrows*) on them. ×500 **b** Electron micrograph through the cell body of the granule cell (*G*) in **a** that displays a small portion of its nucleus and a bulbous process (*arrows*) which is the origin of its basal dendrite. ×13000 **c** Serial section of the same neuron (*G*) showing more of its nucleus and perikaryal cytoplasm that is sparse in organelles. ×8700 **d** Electron micrograph of the basal dendrite (*D*) of this granule cell. The dendrite is apposed by an axon terminal (*T*). ×20000 **e** Enlargement of the axon terminal (*T*) in **d** that forms an asymmetric synapse (*arrow*) with the basal dendrite (*D*) of the granule cell. ×32000

Conclusion

The ultrastructural features of basket cells of the dentate gyrus change in the first two postnatal weeks. At the end of the second week, they reach a mature stage with features similar to those found in basket cells from adults. This is also the stage of an enhanced appearance of the neurotransmitter GABA, and shortly precedes the adult-like appearance of electrical activity of the hippocampus. Therefore, the characteristic ultrastructural features of the cytoplasm and cell nucleus of the basket cells by 16 days may represent the signs of a mature, functionally active stage.

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